Co-location of the multidrug resistance gene cfr and the novel streptomycin resistance gene aadY on a small plasmid in a porcine Bacillus strain

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Sir,

Since its first description in 2000, the multidrug resistance gene cfr has been identified in a number of staphylococcal species. Recent studies, however, identified the cfr gene also in other Gram-positive bacteria, including Enterococcus faecalis and Bacillus spp. However, it should be noted that Bacillus spp. strains in a previous study showed high streptomycin MIC (64 mg/L), indicating the presence of a streptomycin resistance determinant on this small plasmid. In addition, the pBS-03 transformant also exhibited a high streptomycin MIC (64 mg/L), indicating the presence of a streptomycin resistance determinant on this small plasmid.

The sequence of plasmid pBS-03 (accession no. JQ394981) was obtained by primer walking, starting from the amplified cfr gene. Five predicted coding sequences for proteins of ≥100 amino acids were identified in pBS-03 using the GLIMMER software (Figure 1). With a size of 7446 bp, pBS-03 was distinctly smaller than the plasmids pBS-01 (16492 bp) and pBS-02 (16543 bp) previously found in Bacillus spp., and only 2847 bp, including the truncated plasmid recombination/mobilization therapy for this particular pig was not available, the antibiotic usage records of the pig farm in which the Bacillus isolate was collected indicated that a number of antimicrobial agents, including penicillin, florfenicol, gentamicin, streptomycin, apramycin, oxytetracycline and tylosin, had been used for curing or preventing bacterial infections. Sequence analysis of the 16S rRNA of this Bacillus isolate revealed as the best match 96% identity to Bacillus sphaericus, which suggested that isolate BS-03 might belong to a novel Bacillus species. Susceptibility testing of isolate BS-03 showed elevated MICs of chloramphenicol (32 mg/L), florfenicol (32 mg/L), clindamycin (128 mg/L), tiamulin (≥128 mg/L), streptomycin (128 mg/L), kanamycin (≥128 mg/L) and tetracycline (32 mg/L), but showed low MICs of linezolid (2 mg/L), ciprofloxacin (0.5 mg/L), gentamicin (0.5 mg/L) and erythromycin (0.25 mg/L). The MIC of linezolid of 2 mg/L for BS-03 was lower than the MICs observed for cfr-carrying staphylococci (≥4 mg/L), but was consistent with the MIC for the cfr-carrying Bacillus strain BS-03. To date, there is no CLSI-approved breakpoint for linezolid resistance in Bacillus spp. However, it should be noted that >97.5% of the 202 tested Bacillus spp. strains in a previous study showed MICs of linezolid of ≤1 mg/L. Isolate BS-03 was further screened for the genes cfr, fexA and fexB, which are known to confer high florfenicol MICs, using previously described primers. Only the cfr gene was amplified and confirmed by sequencing. Multiple plasmids were extracted from isolate BS-03 using the Qiagen Plasmid Extraction Midi Kit (Qiagen, Hilden, Germany), and subsequently transferred into the Staphylococcus aureus recipient strain RN4220 by electrotransformation with subsequent selection of the transformants on brain–heart infusion (BHI) agar supplemented with 10 mg/L florfenicol. Southern blot analysis showed that the cfr probe hybridized to an ~7.4 kb plasmid (designated pBS-03) in the original isolate and the S. aureus RN4220 transformants (data not shown). The pBS-03-harbouring transformant exhibited at least 4-fold elevated MICs (compared with the ‘empty’ recipient strain) of chloramphenicol (16 mg/L), florfenicol (16 mg/L), clindamycin (128 mg/L), tiamulin (32 mg/L) and linezolid (4 mg/L), which was expected from the carriage of a functionally active cfr gene. In addition, the pBS-03 transformant also exhibited a high streptomycin resistance (64 mg/L), indicating the presence of a streptomycin resistance determinant on this small plasmid.
The gene Δpre/mob and the cfr gene in pBS-03, exhibited >99% nucleotide identity when compared with the sequences of pBS-01 and pBS-02. A 4516 bp segment in pBS-03 containing the Δpre/mob, cfr and most of the complete pre/mob gene presented 99.7% nucleotide similarity when compared with the sequence of pSS-03 (JQ219851), another small cfr-carrying plasmid (7057 bp) widely distributed in staphylococci including *Staphylococcus sciuri*, *Staphylococcus cohnii*, *Staphylococcus arlettae* and *Staphylococcus saprophyticus* of porcine origin. A reading frame for a 376 amino acid Pre/Mob protein downstream of cfr gene in plasmid pSS-03 exhibited 99.2% amino acid identity to the corresponding part of the 390 amino acid Pre/Mob protein of plasmid pBS-03. Further downstream of this pre/mob gene, a reading frame for a 282 amino acid aminoglycoside-6-adenyltransferase conferring streptomycin resistance was detected. This adenyltransferase gene was designated aadY. Its deduced amino acid sequence showed 80.4%–80.1% amino acid identity and 91.3% amino acid similarity to the next related 286 amino acid aminoglycoside 6-adenyltransferases of the *Bacillus cereus* strains Q1 (accession no. ACM15765), AH1272 (accession no. EEL84789), AH1273 (accession no. EEL90107) and R309803 (accession no. EEL78253). Identity between AadY and the 284 amino acid adenyltransferase AadK from *Bacillus subtilis* subsp. *subtilis* strain 168 (accession no. NP_390556) was only 57.8%. The fifth reading frame in pBS-03 coded for a 342 amino acid plasmid replication protein whose deduced amino acid sequence showed only 64.1% identity to the 327 amino acid RepU proteins of the *B. cereus* plasmid pBC16 (NP_043526), as well as the *S. aureus* plasmid pUB110 (accession no. AAA88362) and the RepB of plasmid pAMα1 *E. faecalis* (accession no. NP_863351).

In conclusion, the identification and characterization of plasmid pBS-03 revealed the presence of the gene cfr on a small plasmid from *Bacillus* spp. which also carries the novel streptomycin resistance gene aadY. This observation extends the current knowledge of plasmid-borne resistance genes that may be co-located with cfr and underlines the likelihood of co-selection and persistence of the cfr gene under selective pressure imposed by the use of other antimicrobial agents to which cfr does not confer resistance.

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**Transparency declarations**

None to declare.

**References**


Prevalence of antiseptic resistance genes \(qacA/B\) and specific sequence types of methicillin-resistant \(Staphylococcus aureus\) in the era of hand hygiene

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Sir,

In an attempt to control the spread of methicillin-resistant \(Staphylococcus aureus\) (MRSA) and other multiresistant organisms in hospitalized patients, hand hygiene campaigns have promoted the widespread use of alcohol-based hand solutions containing antiseptic agents such as chlorhexidine and quaternary ammonium compounds. The use of these products, however, has led to increased resistance to these antiseptic agents in MRSA strains.\(^1-5\) \(qacA/B\) are plasmid-borne resistance genes coding for multidrug efflux pumps that confer high-level resistance to chlorhexidine, quaternary ammonium compounds and other antiseptic compounds commonly used in hand hygiene solutions.\(^5\) They are associated with higher MICs and tolerance of these antiseptic agents.\(^1,4,5\) \(qacA/B\) genes have been found in healthcare-associated MRSA (HA-MRSA) isolates from around the world.\(^2-8\) In addition, the prevalence of \(qacA/B\) genes has been associated with particular sequence types (STs) of HA-MRSA.\(^1,3,6\) To our knowledge, the prevalence of MRSA strains harbouring these genes has not been researched in Australia. In this study, we characterize the prevalence of \(qacA/B\) genes in HA-MRSA isolates in our institution spanning 10 years, from 2000 to 2009. We determined whether the national hand hygiene campaign implemented in 2006 had any impact on the prevalence of these genes and, in addition, if any relationship exists between the different STs of HA-MRSA and \(qacA/B\) gene prevalence.

All (total 151) clinically significant unique HA-MRSA isolates from the period 2000–09 from Nepean Hospital, a 490 bed tertiary referral hospital in Sydney, were used in this study. The presence of \(qacA/B\) genes in these isolates was evaluated by real-time PCR using the following primers: \(qacA/B\) forward primer 5′-CTATGCAATAGGATATGGTGT and reverse primer 5′-CCACATAAGTCTCAGCAGAT.\(^7\) The HA-MRSA isolates were categorized into STs using their antibiograms and correlations with pre-existing typing performed on HA-MRSA isolates in our institution by the Australian Group on Antimicrobial Resistance.\(^9\) Quantities of hand hygiene solutions used by the hospital were obtained via the pharmacy database.

A sustained >6-fold increase in antiseptic-containing alcohol-based hand solution use occurred as a result of the hand hygiene campaign (‘Clean hands saves lives’) implemented in 2006 [amounts of 0.5% chlorhexidine gluconate and quaternary ammonium compounds used (average litres per year) were 968 and 150 L before the hand hygiene campaign (2004–06) and 3967 and 2958 L after the hand hygiene campaign (2007–09), respectively].

The yearly prevalence of \(qacA/B\) genes in HA-MRSA isolates from 2000 to 2009 ranged from 65.0% to 94.7% (mean 78.6%). This is higher than that previously found in the UK (8%–26%).\(^5,6\) Europe (63%)\(^3\) and Asia (33%–61%).\(^3,4,7\) but comparable to that found in a recent study in Geneva (79%).\(^8\)

The HA-MRSA isolates in this study comprise two STs: ST239 MRSA-III Aus-2 EMRSA and ST22 MRSA-IV EMRSA-15. The ST239 type was the predominant strain throughout the study period (mean yearly prevalence of ST239 from 2000 to 2009 was 84.3%, range 75%–100%).

Comparing the prevalence of the \(qacA/B\) genes before (2000–06) and after (2007–09) the hand hygiene campaign, we did not find an increase in the prevalence of these genes despite the marked increase in antiseptic hand solutions after 2006 [mean yearly prevalence of \(qacA/B\)-positive MRSA isolates between 2000 and 2006 and between 2007 and 2009 was 78.6% and 73.5%, respectively (\(P=0.53, \chi^2=0.39, df=1\))]. Similarly, there was no change in the incidence of MRSA ST239 [mean yearly prevalence of MRSA ST239 between 2000 and 2006 and between 2007 and 2009 was 85.6% and 78.6%, respectively (\(P=0.37, \chi^2=0.81, df=1\))].

Interestingly, we found a significant association between \(qacA/B\) gene positivity and MRSA type ST239 [94.5% of \(qacA/B\)-positive isolates were ST239 and 88.9% of ST239 isolates were \(qacA/B\) positive (OR = 29.09, CI = 8.93–94.82)]. This relationship between \(qacA/B\) and MRSA ST239 has been suggested in previous studies in the UK and Taiwan;\(^1,3,4\) however, the prevalence